to an enzyme that, in the presence of substrate for said enzyme and tyramide, catalyzes the deposition of tyramide in said cells comprising said intracellular analyte, and contacting the fixed and permeabilized cells with tyramide and a substrate for said enzyme, wherein said enzyme is in a medium containing an agent or agents that reduce non-specific binding to an extent at least equal to the presence of 10% fetal bovine serum;

c) contacting said cells with a detectable label that directly or indirectly binds to tyramide, whereby said cells comprising said intracellular analyte are specifically labeled; and

d) detecting a signal from said cells comprising said detectable label using a flow cytometric device, wherein said signal indicates the presence of said intracellular analyte, and wherein said signal is at least 10-fold greater than a signal obtainable by standard flow cytometry methods in which an immunoglobulin that does not specifically bind said intracellular analyte, and that is isotype/subtype matched to the antibody or fragment thereof of step (b) is used as a negative control.

Comments

In accordance with the telephonic interview on March 16, 2004, proposed amended claim 1 specifies that the antibody or fragment is in a medium that contains an agent or agents that reduces non-specific binding to an extent at least equal to the presence of 10% fetal bovine serum.

Reference to the use of agents that reduce non-specific binding is provided at p.24, lines 24-33. Reference the use of agents that reduce non-specific binding to a greater extent at least equal to the presence of 10% serum is provided in the specification, for example at p.17, line 35 to p.18, line 8.

Because Karkmann and Lollini used 0.5% and 1.0% BSA respectively, the proposed amendment distinguishes the proposed amended Claim 1 over those references, as agreed by the Examiner.

Atty. Dkt. No. 071957-0903

Respectfully submitted,

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By _____

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